A comparison of the effects of calponin on smooth and skeletal muscle actomyosin systems in the presence and absence of caldesmon

Steven J. WINDER, Cindy SUTHERLAND and Michael P. WALSH*
MRC Group in Signal Transduction, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada T2N 4N1

Thiosphosphorylated smooth muscle myosin and skeletal muscle myosin, both of which express Ca\(^{2+}\)-independent actin-activated MgATPase activity, were used to examine the functional effects of calponin and caldesmon separately and together. Separately, calponin and caldesmon inhibited the actin-activated MgATPase activities of thiosphosphorylated smooth muscle myosin and skeletal muscle myosin, calponin being significantly more potent in both systems. Calponin-mediated inhibition resulted from the interaction of calponin with actin since it could be reversed by increasing the actin concentration. Caldesmon had no significant influence on the calponin-induced inhibition of the smooth muscle actomyosin ATPase, nor did calponin have a significant effect on caldesmon-induced inhibition. In the skeletal muscle system, however, caldesmon was found to override the inhibitory effect of calponin. This difference probably reflects the lower affinity of skeletal muscle actin for calponin compared with that of smooth muscle actin. Calponin inhibition of skeletal muscle actin-activated myosin MgATPase was not significantly affected by troponin/tropomyosin, suggesting that the thin filament can readily accommodate calponin in addition to the troponin complex, or that calponin may be able to displace troponin. Calponin also inhibited acto-phosphorylated smooth muscle heavy meromyosin and acto-skeletal muscle heavy meromyosin MgATPases. The most appropriate protein preparations for analysis of the regulatory effects of calponin in the actomyosin system therefore would be smooth muscle actin, tropomyosin and thiosphosphorylated myosin, and for analysis of the kinetic effects of calponin on the actomyosin ATPase cycle they would be smooth muscle actin, tropomyosin and phosphorylated heavy meromyosin, due to the latter's solubility.

INTRODUCTION

Calponin, a calmodulin-, tropomyosin- and actin-binding protein (Takahashi et al., 1986; Takahashi & Nadal-Ginard, 1991), has been implicated in the regulation of smooth muscle contraction (Winder & Walsh, 1990). Calponin is smooth-muscle-specific and is associated with the actin-containing stress fibres in primary cultures of chick embryonic gizzard cells (Gimona et al., 1990) and bovine (Takeuchi et al., 1991) and rabbit (Birukov et al., 1991) aortic smooth muscle cells. Isolated smooth muscle thin filaments contain calponin in addition to actin, tropomyosin and caldesmon (Ngai et al., 1987; Nishida et al., 1990; Winder et al., 1991), and confocal immunofluorescence microscopy indicates that calponin is distributed in single toad stomach smooth muscle cells in the same way as are actin and tropomyosin (Winder et al., 1992a). The concentration of calponin in smooth muscle is equimolar to that of tropomyosin (Takahashi et al., 1986). Purified calponin inhibits the actin-activated myosin MgATPase activity of a reconstituted in vitro contractile system composed of smooth muscle actin, myosin, tropomyosin, calmodulin (CaM) and myosin light chain kinase (MLCK) by \(~80\%\) (Abe et al., 1990; Winder & Walsh, 1990). This inhibitory effect is due to the interaction of calponin with actin and can be prevented by phosphorylation of calponin by either protein kinase C (PKC) or Ca\(^{2+}\)/calmodulin-dependent protein kinase II (CaM kinase II). Phosphorylation prevents the interaction of calponin with actin (Winder & Walsh, 1990). Phosphorylated calponin can be dephosphorylated by a type 2A protein phosphatase, with restoration of its inhibitory effect (Winder et al., 1992b).

Caldesmon has also been implicated in the regulation of smooth muscle contraction (Walsh, 1990; Marston & Redwood, 1991; Sobue & Sellers, 1991). It too can inhibit the actin-activated myosin MgATPase in vitro (Ngai & Walsh, 1984). This protein may act as an actin–myosin cross-linking protein, since the N-terminal domain can interact with the S2 region of smooth muscle myosin and the C-terminal domain with actin (Szpacenko & Dabrowska, 1986; Fuji et al., 1988; Ikebe & Reardon, 1988). The caldesmon–myosin interaction is blocked by phosphorylation of caldesmon by CaM kinase II (Sutherland & Walsh, 1989; Scott-Woo et al., 1990) whereas the caldesmon–actin interaction is blocked by phosphorylation of caldesmon by cdc2 kinase (Mak et al., 1991).

Studies of the inhibition of smooth muscle actin-activated myosin MgATPase by calponin or caldesmon are complicated by the fact that both proteins bind Ca\(^{2+}\)/CaM which is required for myosin phosphorylation; this affects their ability to inhibit the ATPase. We have utilized two ATPase systems to overcome this problem. The first involves smooth muscle myosin which has been thiosphosphorylated; actin activation of its MgATPase therefore no longer requires Ca\(^{2+}\) or CaM. An added advantage of thiosphosphorylated myosin is that, unlike its phosphorylated counterpart, it is resistant to the action of phosphatases (Sherry et al., 1978) which often contain myosin preparations. The second system utilized here involves skeletal muscle actomyosin, even though calponin and caldesmon are not expressed in skeletal muscle, since actin activation of purified skeletal muscle myosin is independent of Ca\(^{2+}\) or phosphorylation; again this permitted the elimination of Ca\(^{2+}\) and CaM from the ATPase reactions. It was also of interest to compare the smooth and skeletal muscle

Abbreviations used: ATP[S], adenosine 5’-O-[γ-thio]triphosphate; CaM, calmodulin; CaM kinase II, Ca\(^{2+}\)/calmodulin-dependent protein kinase II; DTT, dithiothreitol; HMM, heavy meromyosin; MLCK, myosin light chain kinase; PKC, protein kinase C (Ca\(^{2+}\)- and phospholipid-dependent protein kinase).

* To whom correspondence should be addressed: Department of Medical Biochemistry, University of Calgary, 3330 Hospital Drive N.W., Calgary, Alberta, Canada T2N 4N1.

Vol. 288
systems since the affinity of skeletal muscle actin for calponin is 7-8 fold lower than that of smooth muscle actin (Winder et al., 1991). Purified skeletal muscle myosin or heavy meromyosin (HMM) is often used to examine the regulatory and kinetic properties of proteins such as calponin and caldesmon due to the fact that its actin-activated MgATPase activity is unregulated (e.g. Chalovich et al., 1987; Velazquez et al., 1989; Makuch et al., 1991; Marston, 1991). The smooth muscle and skeletal muscle experimental systems therefore enabled us to examine directly the effects of calponin and caldesmon, separately and together, on the actin-activated myosin MgATPases. In addition, the effects of calfponin on the actin-activated MgATPase activity of skeletal muscle and phosphorylated smooth muscle HMMs (soluble, non-filamentous fragments of myosin) were compared. Some of these results have appeared in preliminary form (Sutherland et al., 1990).

**MATERIALS AND METHODS**

Materials

[γ-32P]ATP (10-40 Ci/mmol) was purchased from Amersham Corp. (Oakville, Ontario, Canada). General laboratory reagents used were analytical grade or better and were purchased from Fisher Scientific (Calgary, Alberta, Canada). The following proteins were purified by previously described methods: rabbit skeletal muscle actin (Zot & Potter, 1981), myosin (Persechini & Rowe, 1984) and tropomyosin (Smithie, 1982); chicken gizzard calponin (Winder & Walsh, 1990), caldesmon (Sutherland & Walsh, 1989), myosin (Persechini & Hartshorne, 1981) and actin (Nga et al., 1986); and bovine brain CaM (Walsh et al., 1984). Rabbit skeletal muscle troponin complex was purified as described by Potter (1982) up to and including the ammonium sulphate fractionation step. All protein preparations were > 98% pure, except the troponin complex which was 90.7% pure, as determined by laser densitometry (using an LKB 2202 Ultrascan laser densitometer equipped with an HP3390A integrator) of Coomassie Blue-stained gels of the protein preparations. Rabbit skeletal muscle HMM was prepared by digestion of purified myosin with a-chymotrypsin and purified as described by Margossian & Lowey (1982). Chicken gizzard smooth muscle HMM was prepared by digestion of purified myosin with a-chymotrypsin and purified as described by Seidel (1980).

Enzymic assays

Smooth muscle actin-activated thio phosphorylated myosin MgATPase activity was measured under the following conditions: 20 mM-imidazole/HCl (pH 7.0)/80 mM-KCl/4 mM-MgCl₂/1 mM-dithiothreitol (DTT)/1 mM-EGTA/6 μM-actin/2 μM-tropomyosin/1 μM thio phosphorylated myosin/1 mM-[γ-32P]ATP (~10 000 c.p.m./nmol). Skeletal muscle actin-activated myosin MgATPase activity was measured under the following conditions: 25 mM-Tris/HCl (pH 7.5)/50 mM-KCl/1 mM-DTT/3.5 mM-MgCl₂/0.2 mM-EGTA/3.6 μM-actin/0.57 μM-myosin/1 mM-[γ-32P]ATP (~ 3000 c.p.m./nmol). The reaction mixture (40 μl) was then cooled on ice and slowly diluted with an equal volume of 2 mM-EDTA (pH 7.0). MgCl₂ (1 mM) was added dropwise to a final concentration of 15 mM to precipitate the myosin, which was collected by centrifugation at 15 000 g for 20 min. The pellet was dissolved in 0.5 vol. (20 ml) of 10 mM-Tris/HCl (pH 7.5)/0.2 mM-EDTA/0.3 mM-KCl (solubilization buffer). The dilution/precipitation cycle was repeated twice more to remove CaM and MLCK. The final pellet was dissolved in 0.25 vol. (10 ml) of solubilization buffer and dialyzed overnight versus 2 x 4 litres of the same buffer. The dialysed sample was centrifuged at 2000 g for 5 min to remove insoluble material. Quantitative thio phosphorylation of the myosin was confirmed by the lack of incorporation of [32P]PP into a sample of the preparation under conditions which incorporated 2 mol of Pi/mmol of unphosphorylated myosin. The thio phosphorylated myosin was further characterized by measurements of ATPase activity under the following conditions: 25 mM-Tris/HCl (pH 7.5)/60 mM-KCl/10 mM-MgCl₂/0.1 mM-CaCl₂ or 1 mM-EGTA/1 mM-[γ-32P]ATP (~ 5000 c.p.m./nmol)/1 μM thio phosphorylated myosin/6 μM-actin/2 μM-tropomyosin/75 mM-MLCK/0.6 mM-CaM. Results from a typical preparation are shown in Table 1.

**Electrophoresis**

SDS/PAGE was performed in 7.5–20% polyacrylamide gradient slab gels (1.5 mm thick) with a 5% acrylamide stacking gel, in the presence of 0.1% (w/v) SDS at 36 mA in the.
discontinuous buffer system of Laemmli (1970). Gels were stained in 45% (v/v) ethanol/10% (v/v) acetic acid containing 0.14% (w/v) Coomassie Brilliant Blue R-250, and diffusion-destained in 10% (v/v) acetic acid.

Other methods

Protein concentrations were determined by the Coomassie Blue dye-binding assay (Spector, 1978) using dye reagent purchased from Pierce Chemical Co. (Rockford, IL, U.S.A.) and $\gamma$-globulin as the standard. Myosin, HMM, calponin, caldesmon and CaM concentrations were determined using the following values for the absorbance of a 1% solution with a path length of 1 cm: smooth muscle myosin, 4.5 at 280 nm (Okamoto & Sekine, 1978); skeletal muscle myosin, 5.3 at 280 nm (Margossian & Lowey, 1982); smooth muscle HMM, 6.5 at 280 nm (Chacko & Eisenberg, 1990); skeletal muscle HMM, 6.0 at 280 nm (Margossian & Lowey, 1982); calponin, 11.3 at 277 nm (Winder & Walsh, 1990) caldesmon, 3.3 at 280 nm (Graceffa et al., 1988); CaM, 1.9 at 277 nm (Klee, 1977).

RESULTS

Smooth muscle acto-thiophosphorylated myosin MgATPase

Chicken gizzard smooth muscle myosin was thiophosphorylated and purified as described in the Materials and methods section. The data in Table 1 verify that the MgATPase activity of this thiophosphorylated myosin was markedly increased (9.3-fold) by actin, and that its actin-activated MgATPase activity was independent of Ca$^2+$, CaM and MLCK. Fig. 1 indicates that very little proteolysis occurred during the preparation and purification of thiophosphorylated myosin.

Caldesmon inhibited the acto-thiophosphorylated myosin MgATPase almost completely, with half-maximal inhibition at $\sim 1.5 \mu M$ (Fig. 2a). Caldesmon was significantly less potent, causing only $\sim 65\%$ inhibition at 5 $\mu M$ (Fig. 2b). In the presence of 2 $\mu M$-caldesmon (enough to inhibit the ATPase by 23%), calponin again inhibited the ATPase almost completely, with half-maximal inhibition at 1.3 $\mu M$ (Fig. 2a). On the other hand, caldesmon had no effect on the level of ATPase inhibition induced by 2 $\mu M$-calponin (Fig. 2b); this contrasts markedly with the situation in the skeletal muscle system (see below).

Skeletal muscle actin-activated myosin MgATPase

The inhibitory effect of caldesmon on skeletal muscle actomyosin MgATPase is well known (e.g. Dabrowska et al., 1985; Lim & Walsh, 1986; Chalovich et al., 1987; Smith et al., 1987; Makuch et al., 1991; Marston, 1991). The possibility that calponin and caldesmon may interact functionally in this system was examined by investigating their individual and combined effects on the skeletal muscle actin-activated myosin MgATPase (Fig. 3). As in the case of the smooth muscle system, calponin (Fig. 3a) and caldesmon (Fig. 3b) alone each inhibited the ATPase in a dose-dependent manner, calponin being significantly

![Fig. 1. Characterization of smooth muscle myosin before and after thiophosphorylation](image)

Chicken gizzard myosin (M, 15 $\mu g$) and thiophosphorylated myosin (TPM, 10 $\mu g$) were subjected to SDS/PAGE. HC, heavy chain; LC$_{20}$ and LC$_{17}$, the 20 kDa and 17 kDa light chains respectively.

![Fig. 2. Combined effects of calponin and caldesmon on the smooth muscle acto-thiophosphorylated myosin MgATPase](image)

Smooth muscle actin-activated thiophosphorylated myosin MgATPase activities were measured as described in the Materials and methods section, (a) in the absence (O) and presence (●) of 2 $\mu M$-caldesmon at the indicated concentrations of calponin, and (b) in the absence (O) and presence (●) of 2 $\mu M$-calponin at the indicated concentrations of caldesmon. Each data point represents the mean of three determinations. Control ATPase activity (measured in the absence of calponin and caldesmon) was 100.1 nmol of P$_i$/min per mg of myosin.

Table 1. ATPase activities of smooth muscle thiophosphorylated myosin

<table>
<thead>
<tr>
<th>Reaction conditions</th>
<th>MgATPase rate (nmol of P$_i$/min per mg of thiophosphorylated myosin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca$^{2+}$ Actin CaM MLCK</td>
<td>105.5</td>
</tr>
<tr>
<td>-</td>
<td>103.1</td>
</tr>
<tr>
<td>+</td>
<td>11.3</td>
</tr>
<tr>
<td>+</td>
<td>106.4</td>
</tr>
<tr>
<td>+</td>
<td>108.4</td>
</tr>
<tr>
<td>+</td>
<td>109.7</td>
</tr>
</tbody>
</table>

![Table 1](image)
more potent: at 2 \mu M, calponin induced \sim 75\% inhibition whereas caldesmon induced \sim 50\% inhibition. In contrast to our observations with acto-thiophosphorylated smooth muscle myosin described above, caldesmon had a significant effect on calponin-induced inhibition of the skeletal muscle actomyosin ATPase. At a concentration of caldesmon (2 \mu M) sufficient to cause \sim 50\% inhibition, calponin had no further inhibitory effect (Fig. 3a); in the presence of 2 \mu M-calponin, which induced \sim 68\% inhibition of the skeletal muscle ATPase, caldesmon actually reduced the level of inhibition in a dose-dependent manner to approximately the level observed in the absence of calponin (Fig. 3b).

Calponin-induced inhibition of the skeletal muscle actin-activated myosin MgATPase is due to the interaction of calponin with actin, since it could be prevented by increasing the actin concentration (Table 2). In support of this conclusion, calponin had no direct effect on myosin as shown by its lack of effect on the K' / EDTA-ATPase (at 0.1 M- or 0.3 M-KCl) or CaATPase activities of skeletal muscle myosin in the absence of other proteins (measured as described in the Materials and methods section). The K' / EDTA-ATPase activity (49.0 nmol of P_i/min per mg of myosin at 0.1 M-KCl and 325.3 nmol of P_i/min per mg of myosin at 0.3 M-KCl) was unaffected over the range 0–2 \mu M-calponin; similarly, the CaATPase activity (344.0 nmol of P_i/min per mg of myosin) was unaffected over the same range of calponin concentrations (results not shown).

Table 2. Increasing actin concentration reverses calponin-mediated inhibition of the skeletal muscle actomyosin MgATPase

<table>
<thead>
<tr>
<th>[Actin] (\mu M)</th>
<th>– Calponin</th>
<th>+ Calponin</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>561.2</td>
<td>165.7 (29.5)</td>
</tr>
<tr>
<td>4</td>
<td>661.2</td>
<td>241.6 (36.5)</td>
</tr>
<tr>
<td>6</td>
<td>696.4</td>
<td>286.7 (41.2)</td>
</tr>
<tr>
<td>8</td>
<td>779.1</td>
<td>378.2 (48.5)</td>
</tr>
<tr>
<td>10</td>
<td>676.8</td>
<td>454.1 (67.1)</td>
</tr>
<tr>
<td>12</td>
<td>678.8</td>
<td>629.5 (92.7)</td>
</tr>
</tbody>
</table>

Fig. 4. Inhibition of skeletal muscle actomyosin MgATPase by calponin in the presence of tropomyosin and troponin/tropomyosin

Skeletal muscle actin-activated myosin MgATPase activities were measured as described in the Materials and methods section in the presence of tropomyosin (\square) or tropomyosin, troponin and 0.1 mm free Ca^{2+} (\blacksquare) at the indicated concentrations of calponin. Each data point represents the mean of five or six determinations. Control ATPase activities (measured in the absence of calponin) were 517.9 nmol of P_i/min per mg of myosin in the presence of tropomyosin, and 408.1 nmol of P_i/min per mg of myosin in the presence of troponin/tropomyosin.

Fig. 4 shows that calponin-induced inhibition of skeletal muscle actomyosin ATPase activity, measured in the presence of skeletal muscle troponymosin, is unaffected by the troponin complex, suggesting that the thin filament can accommodate calponin and the troponin/tropomyosin complex or, alternatively, that calponin can displace the troponin complex from the thin filament.

Effects of calponin on smooth muscle and skeletal muscle acto-HMM MgATPases

Phosphorylated rather than thiophosphorylated smooth muscle HMM was used for these experiments, since no myosin phosphatase activity was detected in the HMM preparations. The effects of calponin on the actin-activated MgATPase activities of smooth muscle phosphorylated HMM and skeletal muscle HMM are shown in Fig. 5. Both ATPases were inhibited...
Calponin and caldesmon effects on actomyosin

**DISCUSSION**

Calponin and caldesmon are distinct actin-, tropomyosin- and calmodulin-binding proteins which have been implicated in the regulation of actin–myosin interactions and therefore in the contractile state of smooth muscle (for review, see Walsh, 1991). Vancompernelle et al. (1990) have reported that caldesmon binds to immobilized calponin, but this interaction is disrupted at quite low ionic strength (70 mM-KCl) and therefore may not be of physiological relevance. Since both proteins are associated with the thin filaments in situ (Ishimura et al., 1984; Fürst et al., 1986; Gimona et al., 1990; Birukov et al., 1991; Takeuchi et al., 1991; Winder et al., 1992a), we considered the possibility that they may interact at a functional level. To investigate this possibility, we decided to exploit two experimental systems: (i) thiophosphorylated smooth muscle myosin and (ii) skeletal muscle myosin, since their actin-activated MgATPase activities are unregulated. This obviated the need to add Ca²⁺/CaM, which is required by MLCK for phosphorylation and activation of smooth muscle myosin but is not required in the skeletal muscle system of purified actin and myosin, since its ATPase activity is independent of Ca²⁺ and phosphorylation. The inclusion of CaM in the system would complicate the interpretation of experimental results, since it undergoes Ca²⁺-dependent interactions with both caldesmon (Sobue et al., 1981) and calponin (Takahashi et al., 1986), and high concentrations of CaM can reverse the inhibitory effects of calponin (Abe et al., 1990; Makuch et al., 1991) and caldesmon (Sobue et al., 1982; Dabrowska et al., 1985; Lim & Walsh, 1986; Smith et al., 1987) on the actomyosin MgATPase. Furthermore, Ca²⁺ has been shown to bind directly to calponin (Takahashi et al., 1987), albeit with low affinity (Kₐ = 7 μm) and without an apparent functional effect (Winder & Walsh, 1990).

Using the smooth muscle system, calponin and caldesmon both inhibited acto-thiophosphorylated myosin MgATPase in a dose-dependent manner, but calponin was significantly more potent than caldesmon. Calponin caused half-maximal inhibition of the ATPase at ~1.5 μM and almost complete inhibition at 2 μM. On the other hand, only ~65% inhibition was observed at a caldesmon concentration as high as 5 μM; 50% activity remained at 3.5 μM-caldesmon. Calponin is therefore at least twice as potent as caldesmon in inhibition of the smooth muscle acto-thiophosphorylated myosin MgATPase. In phase smooth muscles, calponin is present at a level of 1 mol/7 mol of actin (Takahashi et al., 1986), whereas the caldesmon content is 1 mol/22–28 mol of actin (Haeberle et al., 1992). On this basis, therefore, calponin would be approx. 8 times more effective than caldesmon in the regulation of the actomyosin MgATPase. However, evidence has been presented suggesting that caldesmon may be localized specifically within the contractile actin domain, which would increase its effective concentration to ~1 mol/14 mol of actin (Small et al., 1986; Marston & Redwood, 1991). Nevertheless, calponin would still be ~4 times more effective than caldesmon. We have suggested previously (Walsh, 1990) that caldesmon may play a structural or organizational role in smooth muscle, rather than a regulatory role, through its ability to cross-link actin and myosin filaments (Ikebe & Reardon, 1988). Our data suggest that calponin may be a bona fide regulatory protein, whereas caldesmon functions to ensure the correct spatial orientation of the contractile filaments in the resting muscle.

The effects of calponin on the skeletal muscle actomyosin system were qualitatively similar to its effects on the smooth muscle system. Quantitatively, however, higher molar ratios of calponin/skeletal muscle actin were required for comparable inhibition. This is presumably due to the fact that the affinity of skeletal muscle actin for calponin is 7–8-fold weaker than that of smooth muscle actin (Winder et al., 1991). Calponin was found to be more potent than caldesmon in inhibition of the actin-activated MgATPase activity of skeletal muscle myosin, as shown above for the smooth muscle system. At 2 μM, calponin inhibited the skeletal muscle ATPase by ~75%, whereas caldesmon inhibited it by ~50%.

When examined together, calponin and caldesmon appeared to function quite independently in inhibition of smooth muscle acto-thiophosphorylated myosin MgATPase activity (Fig. 2). On the other hand, caldesmon had a dramatic effect on calponin-induced inhibition of the skeletal muscle actomyosin ATPase (Fig. 3). Increasing calponin concentrations in the presence of 2 μM-caldesmon caused no further inhibition beyond the level seen with caldesmon alone. Furthermore, in the presence of...
2 μM-calponin, increasing concentrations of caldesmon actually caused a decrease in the degree of ATPase inhibition to approximately the level observed in the presence of caldesmon alone. The differences between the effects of calponin and caldesmon on the smooth and skeletal muscle actomyosin systems indicate fundamental differences in the interaction of calponin with skeletal and smooth muscle actins which warrant further investigation. This is consistent with our earlier observation that the affinity of smooth muscle actin for calponin is 7–8-fold greater than that of skeletal muscle actin (Winder et al., 1991). On the other hand, smooth and skeletal muscle actins have similar affinities for caldesmon (Smith et al., 1987; Velaz et al., 1989).

Makuch et al. (1991) and Marston (1991) also observed dose-dependent inhibition of skeletal muscle actin-activated MgATPase by calponin or caldesmon. However, caldesmon was reported to be more potent than calponin. In the study of Makuch et al. (1991), maximal inhibition (∼80%) occurred at a caldesmon/actin ratio of 1:5 or a calponin/actin ratio of 5:1. Marston (1991) observed maximal inhibition at caldesmon/actin = 1:10 or calponin/actin = 2:5. The reasons for these discrepancies are unclear, since the reaction conditions were quite similar to ours. Since Makuch et al. (1991) obtained the same maximal level of inhibition by calponin or caldesmon, they did not observe the overriding effect of caldesmon on calponin-mediated inhibition that we observed.

Calponin is known to bind in vitro to actin, tropomyosin and Ca²⁺/CaM (Takahashi et al., 1986). We have shown previously that the calponin–actin interaction is responsible for inhibition of the smooth muscle actin-activated myosin MgATPase (Winder & Walsh, 1990). We report here that increasing the actin concentration reverses the inhibition of skeletal muscle actomyosin MgATPase activity by calponin, indicating that calponin inhibits the skeletal muscle system also via its interaction with actin. We observed no effect of calponin on the ATPase activities of skeletal muscle myosin in the absence of actin, consistent with our earlier demonstration that calponin does not interact with smooth muscle myosin coupled to CNBr-activated Sepharose (Winder & Walsh, 1990).

Calponin also inhibited the actin-activated MgATPase activities of both skeletal muscle HMM and phosphorylated smooth muscle HMM. In agreement with the results using intact myosins, calponin was significantly more potent in inhibition of the smooth muscle acto-HMM ATPase. Comparable levels of inhibition of smooth muscle acto-HMM and actomyosin ATPases and of skeletal muscle acto-HMM and actomyosin ATPases were observed at the same calponin/actin molar ratios; although reaction conditions were of necessity rather different. These results indicate that calponin-induced inhibition of actomyosin ATPase activity does not require the light meromyosin domain of the myosin rod, nor does it require that myosin be in a filamentous form.

The major conclusions from this study can be summarized as follows. (i) Calponin and caldesmon both inhibit the actin-activated MgATPase activities of thio phosphorylated smooth muscle myosin and skeletal muscle myosin, with calponin being significantly more potent in each system. (ii) Inhibition is due to the interaction of calponin with actin. (iii) In the smooth muscle system, calponin and caldesmon appear to inhibit the ATPase independently; however, in the skeletal muscle system, caldesmon overrides the inhibitory effect of calponin, possibly due to the lower affinity for calponin of skeletal muscle actin compared with smooth muscle actin (Winder et al., 1991). (iv) Calponin inhibition of the skeletal muscle actomyosin ATPase is unaffected by the presence of the troponin/tropomyosin complex. (v) Calponin also inhibits the acto-phosphorylated smooth muscle HMM and the acto-skeletal muscle HMM MgATPase. The experimental system of choice for analysis of the regulatory properties of calponin is therefore smooth muscle acto-thiophosphorylated myosin. On the other hand, for analysis of the effects of calponin on the kinetics of the actomyosin ATPase cycle, smooth muscle acto-phosphorylated HMM would be the experimental system of choice, due to the solubility of the HMM. Using the latter system, Horiiuchi & Shacko (1991) concluded that the major effect of calponin was on the $V_{max}$ of the acto-HMM MgATPase, with only a small (less than 2-fold) reduction in the $K_{m}$.

This work was supported by a grant to M.P.W. from the Medical Research Council of Canada (M.R.C.C.). M.P.W. is an M.R.C.C. Scientist and Alberta Heritage Foundation for Medical Research Scholar. We are very grateful to Gerry Garnett for word processing.

REFERENCES


Calponin and caldesmon effects on actomyosin


Received 9 April 1992/5 June 1992; accepted 29 June 1992


Vol. 288